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FREIE UNIVERSITÄT BERLIN
Klinik und Poliklinik für Dermatologie
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To whom it may concern
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Ihr Zeichen	Ihre Nachricht	Unser Zeichen	Datum
US 31760			5. Dezember 2002

Re.: US Patent application no. 09/920,392

Declaration

This is to declare that the specific strain reported in the US Patent application no. 09/920,392 has been deposited under the Budapest Treaty and that all restrictions imposed by me as depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent, would satisfy the deposit requirements.

Sincerely

Christos Zouboulis

Prof. Dr. Christos C. Zouboulis

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

The Free University of Berlin
University Medical Center
Benjamin Franklin
Dept. of Dermatology
Hindenburgdamm 30
12200 Berlin

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: The Free University of Berlin Address: University Medical Center Benjamin Franklin Dept. of Dermatology Hindenburgdamm 30 12200 Berlin	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC2383 Date of the deposit or the transfer[1]: 1999-01-13

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 1999-01-14 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH
Address:	Mascheroder Weg 1b D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

U. Weiss

Date: 1999-01-25

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS

FOR THE PURPOSES OF PATENT PROCEDURE

STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT
pursuant to Rule 6.1

To
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-38124 Braunschweig
Federal Republic of Germany

To be filled in by the Depositary Authority

DSMZ-Accession number :

Date culture received:

ANIMAL AND HUMAN CELL CULTURES

THE UNDERSIGNED HEREBY DEPOSITS UNDER THE BUDAPEST TREATY THE CELL CULTURE IDENTIFIED HEREUNDER AND UNDERTAKES NOT TO WITHDRAW THE DEPOSIT FOR THE PERIOD SPECIFIED IN RULE 9.1[1]. THE DSMZ DOES NOT PROPAGATE CELL CULTURES.

I. IDENTIFICATION OF THE CELL CULTURE

Identification reference[2], name of cell line:

SZ95, Immortalized human sebaceous gland cell line
SZ95/K7, clone of the immortalized sebaceous gland cell line to be deposited

Species of origin[3]:

Human, female, facial skin

Hybridoma:

II. CONDITIONS FOR CULTIVATION ( )[4]

Please indicate all necessary conditions including type and % of serum, temperature, gaseous phase, optimal split ratio, etc.:

Dulbecco's modified Eagle's/Ham's F 12 medium (1:1) with N-acetyl-L-alanyl-L-glutamine (2 mM), heat-inactivated fetal calf serum (10%), epidermal growth factor (9 ng/ml), keratinocyte growth factor (9 ng/ml), hydrocortisone (0.4 µg/ml), cholera toxin (10^{-9} M)

37°C, humidified atmosphere with CO_2 (5%)

Optimal split ratio: 1 : 10

Have, until now, any additional supplements (including antibiotics) been used?
If so, give concentrations:

Gentamicin (50 µg/ml)

1 This form may also be used if the undersigned converts into a deposit under the Budapest Treaty the deposit of an organism that he or his predecessor in title has already deposited, outside the Budapest Treaty, with the same depositary institution either before (Rule 6.4(d)) or after the acquisition by that institution of the status of international depositary authority.
2 Number, symbols etc., given to the organism by the depositor.
3 It is strongly recommended that the taxonomic designation and/or scientific description (see under VII.) of the organism be indicated.
4 Mark with a cross if additional information is given on an attached sheet.

III. CONDITIONS FOR LONG TERM STORAGE ( )[4]

Composition of medium:

Dulbecco's modified Eagle's/Ham's F 12 medium (1:1) with N-acetyl-L-alanyl-L-glutamine (2 mM), heat-inactivated fetal calf serum (10%), dimethyl sulfoxide (10%)

Cell concentration: 2×10^6 cells per ampoule (adherent cell culture)

Other recommendations:

IV. KNOWN CONTAMINATION AND PATHOGENICITY ( )[4]

Mycoplasma:		Yes ( )	No (X)	Unknown ( )
Viruses:	Herpes	Yes ( )	No (X)	Unknown ( )
	Hepatitis B	Yes ( )	No (X)	Unknown ( )
	Hepatitis C	Yes ( )	No (X)	Unknown ( )
	HIV	Yes ( )	No (X)	Unknown ( )
Other contaminants:		Yes ( )	No (X)	Unknown ( )

If yes, please specify:

Is the material pathogenic to man or animals:	Yes ( )	No ( )	Unknown (X)

If yes, please specify: pathogenic ( ) allergenic ( ) toxigenic ( ) tumorigenic ( )

THE CELL LINE HAS TO BE HANDLED UNDER THE FOLLOWING LABORATORY CONTAINMENT LEVEL[5]: L1 (X) L2 ( )

[4] Mark with a cross if additional information is given n an attached sheet.

[5] The DSMZ only accepts for deposit organisms which belong to hazard group 1 or 2, according to 'Sichere Biotechnologie: Einstufung von biologischen Agenzien: Viren' (B 004 9/90 ZH 1/344) of the 'Berufsgenossenschaft der chemischen Industrie' and can be handled under the laboratory containment level L1 or L2 according to "Gesetz zur Regelung der Gentechnik" (BGBl. I, pp. 2067-2083 of 21.12.1993).

V. IF THE CELL CULTURE IS GENETICALLY MANIPULATED ( )[4]

1. DATA CONCERNING THE HOST ORGANISM

designation: Human sebaceous gland cell culture (1st subculture)

hazard group: (X) haz. gr. 1 ( ) haz. gr. 2

sensitivities:
resistances:

special properties: Terminal cell differentiation with holocrine secretion, viability up to 3 subcultures

2. DATA CONCERNING THE DONOR ORGANISM

designation: E. coli / SV 40

hazard group: ( ) haz. gr. 1 (X) haz. gr. 2 ( ) haz. gr. 3

description of the cloned DNA fragment:
cloned information: pSV 40 T

size of the cloned DNA (in bp):

( ) complete genome (X) cDNA
( ) subgenomic ( ) synthetic
( ) subgenic

potential risk of the DNA: ( ) pathogenic ( ) tumorigenic
( ) toxigenic ( ) allergenic
(X) no potential risk

3. DATA CONCERNING THE VECTOR

designation: pSVT

derivative of: PBR322-based construct containing the sequences encoding the
host specificity: transforming protein SV 40 large T protein

resistances:

plasmid/virus size (incl. insert):

promoters: Rous Sarcoma Virus long terminal repeat

additional reading frames:

own infectiosity:	( ) yes	(X) no
mobilisable plasmid:	( ) yes	(X) no
own transfer system:	( ) yes	(X) no
transfer by endogenous helper viruses:	( ) yes	(X) no

4. DATA CONCERNING THE GENETICALLY MANIPULATED ORGANISM[5]

special properties:
(e.g. production of ...; use as ...-vector etc.) Immortalized cell line showing morphologic, phenotypic and functional characteristics of non-transfected human sebaceous gland cells

foreign DNA: (X) unknown ( ) episomal ( ) chromosomally integrated

potential risk: ( ) pathogenic ( ) tumorigenic
( ) toxigenic ( ) allergenic
( ) no potential risk
please indicate why:
Pure cell culture, no viruses, PBR322 derivative, uknown tumorigenic potential

According to the regulations of the GenTG[6] the DSMZ can only accept genetically manipulated, potentially pathogenic organisms for deposition when a copy of the permit issued by the competent authority (or by an equivalent national biological safety commission)

[4] Mark with a cross if additional information is given on an attached sheet.
[5] The DSMZ only accepts for deposit organisms which belong to hazard group 1 or 2, according to 'Sichere Biotechnologie: Einstufung von biologischen Agenzien: Viren' (B 004 9/90 ZH 1/344) of the 'Berufsgenossenschaft der chemischen Industrie' and can be handled under the laboratory containment level L1 or L2 according to "Gesetz zur Regelung der Gentechnik" (BGBl. I, pp. 2067-2083 of 21.12.1993).
[6] GenTG = Gesetz zur Regelung der Gentechnik (German law for the regulation of genetic engineering)

VI. SCIENTIFIC DESCRIPTION[7] (X)[4]

The SZ95 cell line derived by transfection of human facial sebaceous gland cells (1st subculture, female donor) with a PBR-322-based plasmid containing the coding region for the SV 40 large T antigen. The resulting cells have been passaged over 50 times to date, have been cloned, and show no signs of senescence after 3.5 years in vitro. The immortalized cells, termed SZ95, express the SV 40 large T antigen and present an hyperdiploid-aneuploid karyotype with a modal chromosome number of 64.5. SZ95 cells show morphologic, phenotypic and functional characteristics of normal (non-transfected) human sebaceous gland cells. From the clones investigated, the clone designated SZ95/K7 is sent for deposition.

VII. ADDITIONAL DATA[8] ( )[5]

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VIII. FATE OF THE CULTURE AFTER THE PRESCRIBED DURATION OF STORAGE[10]

a) The culture is to be transferred into the publicly available collection of the DSMZ	( ) yes	(X) no
b) The culture is to be handed back to the depositor against a fee	( ) yes	(X) no
c) The culture is to be destroyed by the DSMZ	(X) yes	( ) no

IX. DEPOSITOR[11]

Name: Dr. Christos C. Zouboulis Signature:

Address: Department of Dermatology Date: 25.11.1998
University Medical Center Benjamin Franklin
The Free University of Berlin
Hindenburgdamm 30
12200 Berlin

[4] Mark with a cross if additional information is given on an attached sheet.
[7] It is strongly recommended to indicate the scientific description and/or proposed taxonomic designation (see I.) of the microorganism.
[8] If desired name and address of the inventor(s) might be recorded here.
[9] Mark with a cross if additional information (other than the information referred to in footnote 5 is given on an attached sheet, such as the source of the microorganism, the name(s) and the address(es) of any other depositary institution(s) with which the microorganism has been deposited, or the criterion used when drafting the proposed taxonomic designation (The supplying of such information is optional).
[10] The culture is to be stored for a period of at least five years after the most recent request for the furnishing of a sample of the deposited organism and, in any case, for at least 30 years after the date of deposit (Rule 9.1). The above regulation is valid till there will be binding jurisdiction.
[11] This Deposition Form is the contract between the depositary and the depositor. Therefore it must be signed by the depositor. In case of a legal entity the signatures of two representatives, officially nominated by this entity, are required. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on behalf of the legal entity should accompany the signature(s). Unless otherwise agreed, the undersigned is the correspondent of the DSMZ.

SZ95 cell line – clone SZ95/K7 (passage 50)

Immortalized human sebaceous gland cell line transfected with a PBR-322-based plasmid containing the coding region for the SV 40 large T antigen

Source:
Facial skin, 87-year-old female

Time in culture:
- More than 3½ years
- Over 50 passages (November 1998)

Medium:
Dulbecco's modified Eagle's (DMEM)/Ham's F 12 medium (1:1) with N-acetyl-L-alanyl-L-glutamine 2 mM, heat-inactivated fetal calf serum (FCS) 10 %, epidermal growth factor (EGF) 9 ng/ml, keratinocyte growth factor (KGF) 9 ng/ml, hydrocortisone 0.4 µg/ml, cholera toxin 10^{-9} M, gentamicin 50 µg/ml

Cytology:
- Epithelial, polymorphous morphology
- Different cell sizes of 3.2 to 3.25-fold during proliferation and 5 to 6-fold at confluence
- Keratin cytoskeleton
- Positive for SV 40 large T antigen

Growth potential:
- Immortality, split at subculture: 1:10
- Population doubling time 14.5 - 35 h depending on the initial cell density

Differentiation:
Keratin expression: 7, 13, 16, 19
- Proteins of the polymorphous epithelial mucin group: Human epithelial sialo-mucin (MAM-6), human milk fat globulin-1 (HMFG-1), human milk fat globulin-2 (HMFG-2), Thomsen-Friedenreich antigen, Mucin-like carcinoma-associated antigen (MCA), epithelial membrane antigen (EMA), sebaceous gland antigen (OM-1)
- 5α-reductase type 1
- Lipid synthesis including triglycerides and free fatty acids, as well as the sebaceous lipids squalene and wax esters (clone SZ95/K7)

Functional characteristics:
- Reduced growth and lipid synthesis under serum-free conditions
- Retrieval of cell proliferation rates after addition of 5α-dihydrotestosterone (5α-DHT) (clone SZ95/K7)
  Inhibition of cell proliferation by retinoids (13-*cis* retinoic acid and all-*trans* retinoic acid but not acitretin) (clone SZ95/K7)

Karyotype:
Hyperdiploid-aneuploid, SZ95: 60 to 67 chromosomes (median 64.5)
clone SZ95/K7: 60 to 69 chromosomes (median 63.5)